DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ANTIGENS
Abbott HIVAG-1 Monoclonal
Direct Serum Assay

I. PRINCIPLE

The Human Immunodeficiency Virus Type 1 (HIV-1) is recognized as the etiologic agent of acquired immunodeficiency syndrome (AIDS). The virus is transmitted by sexual contact, exposure to infected body fluids or tissues, and from mother to fetus or child during perinatal period. After exposure to the virus, HIV-1 infection is characterized by an early period of antigenemia in which HIV-1 antigens (Ag) are detectable in blood. In most individuals the antigen level becomes undetectable for a period of time; late in disease, increasing failure of the immune system and increasing levels of virus may again result in detectable levels of antigen. One of the viral components in blood during antigenemia is the core protein, p24, the major internal structural protein of HIV-1.

The Abbott HIVAG-1 Monoclonal Assay is an enzyme immunoassay (EIA) developed for detection uncomplexed HIV-1 p24 antigen. The Abbott HIVAG-1 Monoclonal Assay is a "sandwich" solid phase immunoassay that uses a polystyrene bead coated with human monoclonal antibody (Ab) to HIV-1 p24. If present, the viral antigen binds to the antibody coated bead. Following a wash step, rabbit antibody to HIV-1 is then incubated with the beads and binds to the HIV-1 Ag. Goat antibody to rabbit IgG conjugated with horseradish peroxidase (anti-Rabbit IgG: HRPO) is incubated with the beads and binds to rabbit antibody. Next o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the beads, and after incubation, a yellow-orange color develops in proportion to the amount of HIV-1 antigen bound to the bead. The quantity of HIV-1 antigen in a specimen is determined by comparing its absorbance with that of known a HIV-1 p24 antigen standard curve.

II. SPECIMEN REQUIREMENTS

Serum or plasma collected in acid-citrate-dextrose (ACD), citrate-phosphate-dextrose with adenine (CPDA-1), EDTA, sodium citrate or heparin may be used and should be tested as soon as possible following collection. If the situation limits the ability to test the sample quickly, the specimen can be held in refrigeration (2-4^{0}C) for a maximum of 7 days. If the period of time will be greater, the sample can be held at -20^{0}C to -85^{0}C for long term storage.

Remove the serum from the clot, or plasma from the red cells as soon as possible to avoid hemolysis.

Specimens containing particulate matter may give inconsistent results. Such specimens should be clarified by centrifugation prior to assay.

Heat-inactivated specimens or specimens with obvious microbial contamination are unacceptable.

Avoid subjecting specimens to repeated freeze thaw cycles.

Bring all specimens to room temperature (15-30^{0}C) prior to assay.

III. REAGENTS

A. The following reagents are included in the Abbott HIVAG-1 Monoclonal Kit. Kit reagents may be used at room temperature or cold unless otherwise stated in reagent preparation.

1. HIV-1 (Human) Monoclonal Antibody-coated Beads. Store at 2-8^{0}C. Note manufacturer's outdate. Replace desiccant after use and cap tightly for storage.

2. Antibody to HIV-1 (Rabbit). Store at 2-8^{0}C. Note manufacturer's outdate.

3. Anti Rabbit IgG Conjugate (Rabbit). Store at 2-8^{0}C. Note manufacturer's outdate.

4. Diluent for OPD tablets. Store at 2-8^{0}C. Note manufacturer's outdate.

5. OPD Tablets (o-Phenylenediamine-2HCL). Store at 2-30^{0}C. Note manufacturer's outdate. Prepare OPD Substrate Solution fresh for each assay as follows:

   a. Bring OPD tablets and diluent to room temperature before use. Do not open tablet bottle until it reaches room temperature.

   b. Within 5-60 minutes of use, prepare sufficient OPD Substrate Solution by dissolving the OPD Tablets in the OPD Diluent. See chart below.

No of Beads	Tablets	Diluent (mL)
13	1	5
28	2	10
43	3	15
58	4	20
73	5	25
88	6	30
103	7	35

c. Just prior to dispensing, swirl the container gently to obtain a homogenous solution.

Note: Do not use an OPD tablet that is broken. Transfer tablets with non-metallic forceps into a metallic free bottle. OPD Substrate solution must be used within 60 minutes. OPD Substrate must not be exposed to strong light.
Do not cap tightly while dissolving.

6. Specimen Diluent containing Triton X-100. Store at 2-8^{0}C. Note manufacturer's outdate.

7. Stop Solution (1N H_2SO_4). Store at 2-30^{0}C. Note manufacturer's outdate.

8. Negative Control. Store at 2-8^{0}C. Note manufacturer's outdate.

B. Reagents required but not provided:

1. 5% Hypochlorite solution (household bleach) diluted 1/10, or other appropriate disinfectant.

2. Deionized or distilled water.

C. Standards and controls for the assay provided by the Virology Quality Assurance Laboratory (VQA):

1. VQA SQC (Serum Quality Control). A set of five concentrations. Store at -80^{0}C.

a. Just prior to set up, thaw 1 vial of each of the 5 concentrations.

b. Mix well and use.

IV. SUPPLIES AND EQUIPMENT

Lab coat
Gloves
Reaction Trays (Abbott)
Assay Tubes (Abbott)
Cover seals (Abbott)
Micropipet(s) capable of delivering 20, 50, 180 µL volumes
Precision pipettes, or similar equipment to deliver 200 µL, 300 µL ,and 1 mL
Disposable pipette tips suitable for the above pipettes
Disposable serological pipettes

Disposable reagent reservoirs
Vortex mixer
Centrifuge
Commander Dynamic Incubator (Abbott) capable of 40 ± 2^0C with rotation
Graduated cylinders and beakers
12 x 75 mm tubes
Washing device for washing beads such as Quickwash® or Pentawash® II with vacuum source and a double trap for retaining the aspirate, and capable of delivering a total rinse volume of 4-6 mL per well
Quantumatic™ or Quantum Analyzer™ or spectrophotometer able to read absorbance at 492 nm
Bead dispenser (Abbott)
Non-metallic forceps
Metal free container for OPD Substrate Solution can be plastic or acid washed glassware

V. PROCEDURE

1. Create an EIA template in the virology data-management software (see software manual).

2. In an Abbott EIA reaction tray, dispense 50 µL of Specimen Diluent to each well.

3. Transfer 200 µL of the SQC, and patient specimens to the corresponding wells in the reaction tray.

4. Carefully dispense 1 bead to each testing well.

5. Cover the reaction tray using an adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at 40 ± 2^0C for 1 hour ± 5 minutes using an Abbott Dynamic Incubator with rotation.

6. Remove the cover from the reaction tray and discard. Wash each bead.

7. Add 200 µL Antibody Solution to all testing wells. Cover the reaction tray using a new adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at 40 ± 2^0C for 1 hour ± 5 minutes using an Abbott Dynamic Incubator with rotation.

8. Remove the cover from the reaction tray and discard. Wash each bead.

9. Add 200 µL of Conjugate Solution to all testing wells. Cover the plate using a new adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at 40 ± 2^0C for 1 hour ± 5 minutes using an Abbott Dynamic Incubator with rotation.

10. Remove the cover from the reaction tray and discard. Wash each bead.

11. Immediately transfer the beads to a properly identified box of assay tubes. Add 300 µL of OPD substrate solution into 2 empty tubes (substrate blanks) and then into all tubes containing a bead. Cover the tubes to prevent exposure to intense light. Incubate at room temperature (15-30^{0}C) for 30 $\pm$ 2 minutes.

12. Add 1 mL of Stop Solution to all tubes.

13. Blank spectrophotometer with one of the substrate blank tubes at 492 nm. Read the absorbance of each tube at 492 nm within 2 hours of the addition of stop Solution.

VI. CALCULATIONS

The HIV-1 p24 antigen concentrations may be generated from a virology data-management software program developed for the Division of AIDS (DAIDS) to ensure data integrity of both QA and test specimens. A weighted linear least squares method using the VQA SQC concentrations is used to estimate HIV-1 p24 antigen concentration.

VII. QUALITY CONTROL

The absorbances obtained form the spectrophotometer may be transferred into the virology data-management software program. The software program incorporates two QC check programs, Cum Sum and Levy Jennings. These two programs review the absorbance of the VQA SQC and compare them to established standard deviation ranges. These ranges are determined by the testing laboratory and is reflective of values unique to each laboratory. The software will flag values that fall outside of the laboratory's standard deviation range. The technician must determine the significance of the out of range QC and resolve the situation.

VIII. PROCEDURAL NOTES

When dispensing beads, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells of the reaction tray. Beads may also be dispensed using plastic forceps.

Do not splash liquid when tapping trays.

When transferring beads from wells to assay tubes, align inverted rack of orientated tubes over the reaction tray. Take care that well A1 aligns with tube A1! Press the tubes tightly over the

wells and invert tray and tubes together so the beads fall into corresponding tubes. Blot excess water from the top of the tube rack.

Dispense acid in same tube sequence as OPD Substrate Solution.

IX. REFERENCES

Abbott HIV-1 Antigen Assay package insert and all references within.